Original Research Article

Molecular characterization of Indoor air Microorganisms of a Model Primary Health Care in Port Harcourt, Rivers State, Nigeria.

ABSTRACT

Aims: The quality of indoor air in some government health institutions in Port Harcourt was evaluated with a view to determining the level of microbial contamination and to carry out molecular identification of the microorganism in the indoor air.
Study design: Experimental analysis of the Indoor air of frequently used wards.
Place and Duration of Study: Model Primary Health Care in Port Harcourt, Rivers State, Nigeria, between January 2018 and March 2018.
Methodology: The open plate, impingement technique was used in this study. In this technique, nutrient agar, and mannitol salt agar plates were exposed to the ambient air of the sites studied in duplicates for 15 minutes so as to enumerate and identify the heterotrophic bacteria and staphylococcal species.
Results: The mean heterotrophic bacteria and staphylococcal loads for the morning period of the Health centre ranged from 1.8×10^3 to 4.1×10^3 and 8.6×10^2 to 2.5×10^3 Cfu/m^3, respectively, while the mean heterotrophic bacteria and staphylococcal load for the evening session ranged from 3.5×10^3 to 5.1×10^3 and 8.9×10^2 to 1.7×10^3 Cfu/m^3, respectively. *Bacillus cereus, Chryseobacterium* sp*, Proteus mirabilis, Staphylococcus aureus,* and *Staphylococcus epidermidis* were the bacteria isolated.
Conclusion: The bacterial isolates in this study could contaminate hospital equipment and could cause nosocomial infections if not properly managed. Also, the bacterial loads were very high and exceeds suggested limit for air microflora.

Keywords: Indoor air, Model health care, Bacteria, Staphylococcus sp, Molecular characterization.

1. INTRODUCTION

Indoor air is the air that is within a building. The air within a building that aids comfort and good health of the occupants is referred to as the indoor air and the quality of the indoor air is influenced by contaminations from microbial sources, gases as well as other particles which could lead to poor health conditions [1].

There is a strong connection between the human health and a healthy environment [2]. This could imply that the type of microorganism in the air inside a building could affect or influence the health of the persons within that building and that the nature or type of the organism and the number of the organisms could be related to the persons within the building and the type of activities carried out [3]. Biological sources which contaminates the air could be bacteria, fungi, pollen, viruses and mites [4, 5]. For good health and wellbeing, all living humans and animal need clean air. However, due to urban development, the air is continuously polluted. Urban ambient air is more polluted than overall atmosphere, due to high density of human population and their activities in urban areas [6]. Physical, chemical and biological factors can alter or change the indoor air quality and the physical factors

include a range of issues from temperature, humidity and air movement to dust, lighting and noise, while chemical factors include pollutants arising from paint, carpets, furniture, environmental tobacco smoke, cosmetics and drapes. For the biological factors, microorganisms play the main role, because the inhalation of bacterial, fungal and micro algal spores can cause an allergic reaction [7]. A poor indoor air quality can cause a variety of short-term and long-term health problems including allergic reactions, respiratory problems, eye irritation, sinusitis, bronchitis and pneumonia [1]. Fungal flora can be hazardous for health, particularly in rooms with heating, ventilation and air conditioning systems in place [8]. Biological contamination of indoor air is mostly caused by bacteria, moulds and yeast. They can be dangerous as pathogenic living cells but they can also secrete some substances harmful for health. These are different kinds of toxic metabolism products, for example mycotoxins [9]. Information regarding the quality of indoor air in government model primary health care in Rivers State is scanty. Also, different studies of indoor air in Rivers State have characterized the microorganisms using colonial/morphological characteristics and biochemical reactions. This study therefore was aimed at investigating the indoor air quality as well as characterizing the microorganisms using both cultural and molecular methods.

2. Material and Method

2.1. Description of Study Area

The study area was the Model Primary Health Centre, Rumuigbo. The model primary health care centre, Rumuigbo is located in Rumuigbo along the Rumuokoro high way immediately after the Obi Wali round about in Obio/Akpor Local Government Area, Rivers State. Its coordinates were 4.850^{o}N and 6.991^{o}E. There are about eight wards within the primary health centres out of which only four (namely the children ward, Outpatient ward, Injection room and the Post-natal ward) are constantly used while the other wards were not utilized as at the time of this study.

2.2. Indoor Air Sampling

The Koch's sedimentation method also known as the "settling plate" or "passive" air technique described by previous studies [3, 10, 11] was adopted. This technique involved exposing Petri plates containing growth medium to the atmosphere of the place under study for a given time of which 15 minutes was used in this study. Four wards which includes the children ward, outpatient ward, post-natal ward and the injection room of the health centre was studied. The indoor air was sampled for two durations of the day (i.e. morning and evening).

2.3. Microbiology of Indoor Air

2.3.1. Enumeration and Isolation of Indoor Bacteria

Freshly prepared sterile nutrient agar (NA) and Mannitol salt agar plates in duplicate were exposed to air at the different sampling sites for about 15 minutes to allow air microflora within the wards to settle on the surface of the medium by gravity. The plates were kept about 1m above ground level to eliminate possible contamination and aid quick settling of microbial particles. The plates were covered and transported to the microbiology laboratory of the Rivers State University and incubated for 24 hours at 37^{o}C. Counts were made for plates that showed significant growth at the end of incubation as described by Amadi *et al.* [12].

2.3.2. Estimation of the Colony Forming Units (Cfu)

The colonies from each plate were estimated using the Koch's sedimentation formula as described by previous studies [3, 10, 11].

$$A = \frac{a \times 10000}{0.2 \times \pi r^2 \times t}$$ ..... equation.

Where:
A = CFU (colony forming unit)
a= average number of colonies
r = radius of Petri dish
t = time (minute) of exposure of the plate

2.3.4 Isolation of bacteria

Discrete colonies on the different media plates were picked and inoculated onto freshly prepared nutrient agar plates for bacteria purification. Pure cultures of the isolates were obtained by streaking the isolates on freshly prepared medium until it was ascertained that there were no contaminants.

2.3.5. Maintenance of Pure Cultures

Pure cultures of bacteria were stored in duplicate in 10% (v/v) frozen glycerol suspensions in the refrigerator [12] and on nutrient agar slants. This served as stock cultures used for further identification procedures.

2.3.6. Characterization and Identification of Bacterial Isolates

Methods of characterizations employed were colour, shape, texture, odour, and microscopy under an oil immersion light microscope. Biochemical tests adopted include motility, catalase test, growth on blood agar, haemolysis test and coagulase test, citrate utilization and sugar fermentation tests. The procedures were carried out as described by Cheesbrough [13]. Isolates were further confirmed using the Bergy's manual of determinative Bacteriology [14] before subjecting them to the molecular base method of identification.

2.3.7. Molecular-Based Technique

DNA extraction (Boiling method)

The boiling method as described by previous studies [15, 16] with slight modification was used in extraction of bacteria DNA. Twenty-four (24) hours old cultures of test isolates were transferred in to Luria Bertani (LB) medium and incubated for 24 hours. After incubation, five milliliters of the turbid overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000rpm for 3 min. The cells were re-suspended in 500µl of normal saline and heated at 95^{o}C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml micro centrifuge tube and stored at -20^{o}C [17].

2.3.7.1. Quantification of DNA

The Nanodrop 1000 spectrophotometer was used to quantify the extracted DNA.

2.3.7.2. Amplification of the 16S rRNA

This was carried out according to the methods of Saitou and Nei [18]. The 27F and 1492R primers on ABI 9700 Applied Biosystems thermal cycler in a total volume of 25µl for 35 cycles were used to amplify the 16s rRNA of the rRNA genes of isolates. The PCR mix was composed of the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl). The forward and reverse primers at a concentration of 0.4M and the extracted DNA representing the template. The conditions of the PCR were adjusted: initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator.

2.3.7.3. Sequencing and Phylogenetic Analysis

The BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa was used in sequencing. Phylogenic analysis was carried out by editing resulting sequences with the aid of the bioinformatics algorithm Trace edit tool having downloaded similar sequences from the National Center for Biotechnology Information (NCBI) data base using BLASTN. Downloaded sequences were aligned using ClustalX and the evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 [18]. The bootstrap consensus tree inferred from 500 replicates [17] was taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969).

3. RESULTS AND DISCUSSION

The mean counts for the total heterotrophic bacteria and staphylococcal counts of the various wards is presented in Figures 1 and 2, respectively. The mean counts of the heterotrophic bacteria for the morning period ranged from 1.8×10^3 to 4.1×10^3; while the evening counts ranged from 3.5×10^3 to 5.1×10^3 cfu/m^3. It was observed that the highest bacterial load for both the morning and evening period was recorded in the Outpatient ward (Fig. 1). Furthermore, the outpatient ward had the highest viable bacterial load followed by the children ward. The injection ward had the least viable bacteria counts for both periods (Fig 1). The low bacterial loads recorded in the injection ward of the Health Centre could be attributed to the purpose for which it is used for. The injection ward is the ward that is set aside to administer injections and it was observed during the study that patients were not much in this ward as compared to other wards. The high bacterial load in the outpatient ward was expected as it served different purposes such as sensitization or sharing of health tips, reception, sample collection points and site for dispensing drugs. Thus, the outpatient ward was always crowded. Statistically, there was no significant difference (*P>0.05*) in the total heterotrophic counts between the children and postnatal ward while there was a significant difference between the injection, outpatient ward and children ward.
The staphylococcal load ranged from 8.6×10^2 to 2.5×10^3 and 8.9×10^2 to 1.7×10^3 cfu/$m^3$for the morning and evening session, respectively. The postnatal ward had the highest staphylococcal load followed by the outpatient ward in the evening period (Fig 2). Despite the high staphylococcal counts recorded in the various study sites, there was no significant difference at P>0.05 for the morning session and the evening period.

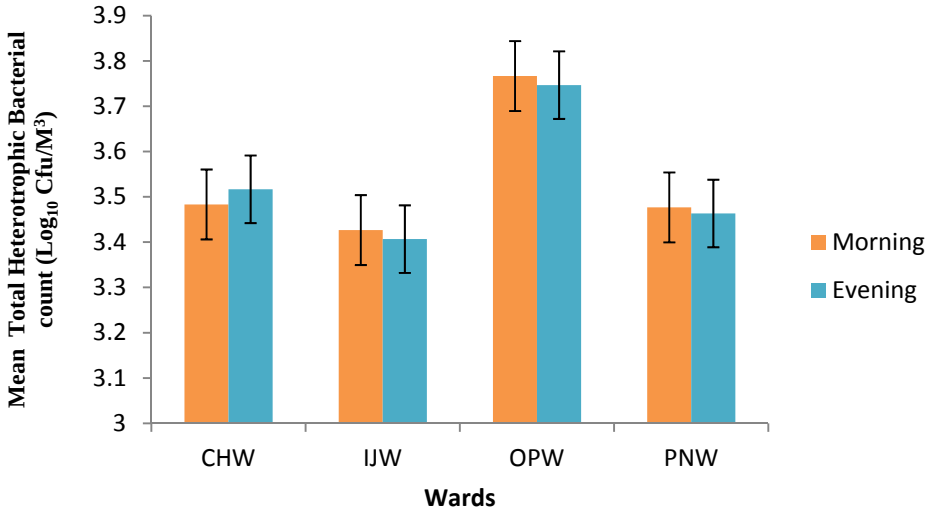

Fig 1. Total heterotrophic bacteria of morning and evening sessions of the Rumuigbo health centre

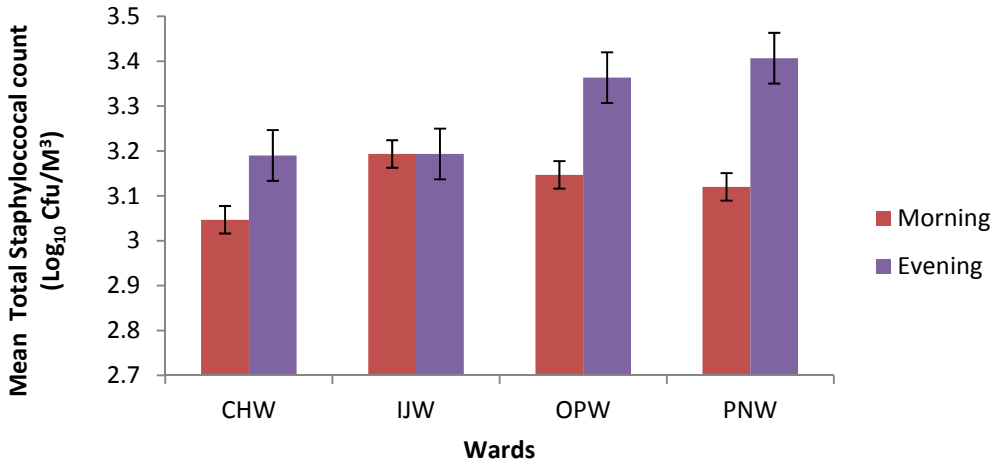

Fig 2. Staphylococcal load of morning and evening sessions of the Rumuigbo health centre

Apart from the heterotrophic bacterial load of the children ward, the staphylococcal load of the postnatal and outpatient ward which were higher in the evening period, all other microbial populations (heterotrophic bacterial, and staphylococcal loads) of the respective wards were more in the morning period. The findings in this current study of the microbial population greatly deviates from the result of a recent study on the evaluation of indoor air for bacteria organisms and their antimicrobial susceptibility profiles in a government health institution by Wemedo and Robinson [3] who reported that the microbial loads in the evening period were higher than those of the morning period. The health Centre does not have staffs on shift during the evening period which makes the area vacant as nurses including staffs and patients vacate the building. The high bacterial load in the evening period of the children ward could be attributed to the presence of more children as well as the continuous movement of clinicians and parents within the ward. High microbial load during the evening period has been reported in previous studies [3, 19]. The high microbial populations during the sampling periods might have been influenced by a number of factors including the number of persons in the wards, ventilation methods as well as activities being carried out within this space. Studies have revealed that the influx of persons as well as activities within those spaces is responsible for high microbial load as well as the type of microorganisms within the indoor air [3, 11, 20]. During the study, it was observed that the health centre relied on mechanical and natural ventilation during the peak of work activities, and the mechanical ventilators which were electrically powered were not consistent due to power fluctuations. The evening period were also ventilated mechanically (electric ceiling and standing fans) and naturally via open windows. This could also be a considerable factor for the fluctuations of microbial load observed in the various study sites. Also, the health centres were sited along busy roads and accommodations for staffs were provided within the hospital area. Thus, activities of the indwellers could contribute to the increased microbial load after work activities. Other scholars had revealed that direct relationship exist between ventilation and indoor levels of bacteria and fungi. Assertions were made stating that good ventilation causes a reduction in microbial load [20].

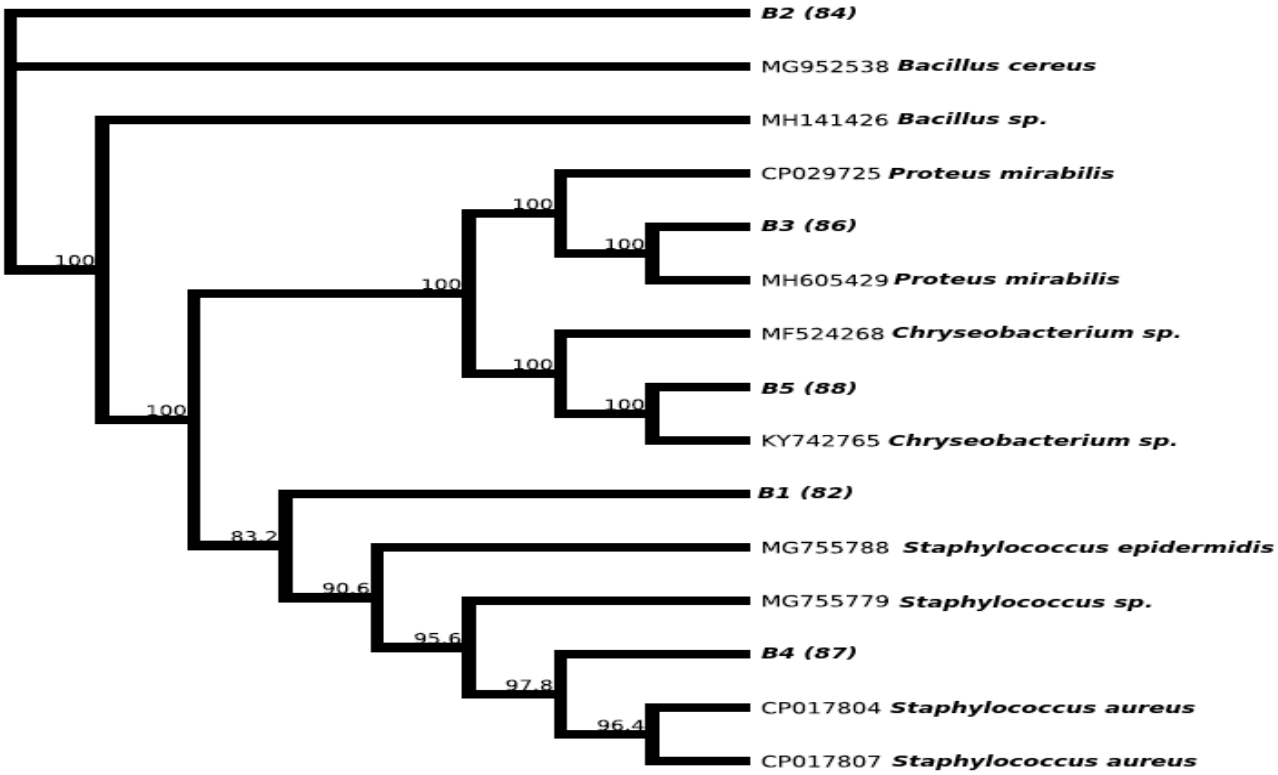

Fig 3: Phylogenic tree of the various isolates showing evolutionary distances

During the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) data base of the extracted 16S rRNA sequence of the isolates, an exact match was found. The 16S rDNA of the isolates B2, B3, B5, B1 and B4 showed a 99% percentage similarity to *Bacillus cereus*, *Proteus mirabilis*, *Chryseobacterium* sp, *Staphylococcus epidermidis* and *Staphylococcus aureus*. The evolutionary distances computed using the Jukes-Cantor method were in conformity with the phylogenetic placement of the isolates within the *Bacillus, Proteus, Chryseobacterium,* and *Staphylococcus* sp and revealed closely relatedness to *Bacillus cereus, Proteus mirabilis, Chryseobacterium, Staphylococcus epidermidis* and *Staphylococcus aureus* (mg952538, mh605429, ky742765, mg755788 and mg755779), respectively than other species) (Figure 3).

In the primary health centre, *Bacillus, Chryseobacterium* and *Staphylococcus* species were isolated in all the study sites (wards) while *Proteus mirabilis* was isolated in the entire ward except in the outpatient ward. *Staphylococcus* species, *Bacillus* species, and *Clostridium* species are the most prevalent bacterial genera that are found within the indoor air [21]. The frequency of occurrence of bacterial isolates in the children ward revealed that *Bacillus* was most predominant (20%) followed by *Chryseobacterium* (17.4%) and *Proteus* (11.1%) while *Staphylococcus* was the least (7.1%). In the outpatient ward, *Chryseobacterium* species had the highest frequency of occurrence (43.5%) followed by *Staphylococcus* species (35.7%) while *Proteus mirabilis* did not occur (Fig 3). The frequency of occurrence of *Proteus* species in the postnatal ward was higher than other bacterial isolates and was the second most

predominant in the injection ward (38.9%) which was dominated by *Staphylococcus* species (42.9%) as shown in Fig 4. *Chrysobacterium* species which are gram negative rods are ubiquitous with major occurrence in soil, fresh and marine water including chlorinated water. As an environmental contaminant, it can colonize sinks and taps and also serves as reservoirs for infections within health care environments. Infections through contaminated medical equipment such as respirators, mist tents and humidifiers, for new babies and adults with compromised immune system have been reported [22, 23]. Thus, there prevalence in the sampling sites of the study locations could be attributed to the flow of air current from the outside environment or through the use of contaminated water to clean floor surfaces or the use of contaminated medical instrument. *Chryseobacterium* species are transient flora [23] so discharge by human into the environment could only be by persons infected with the pathogens. Previous studies have reported it to be associated with infections such as endocarditis, wounds, skin including soft-tissue infections [24, 25, 26]. Thus, there prolonged prevalence in this study site could result to infections.

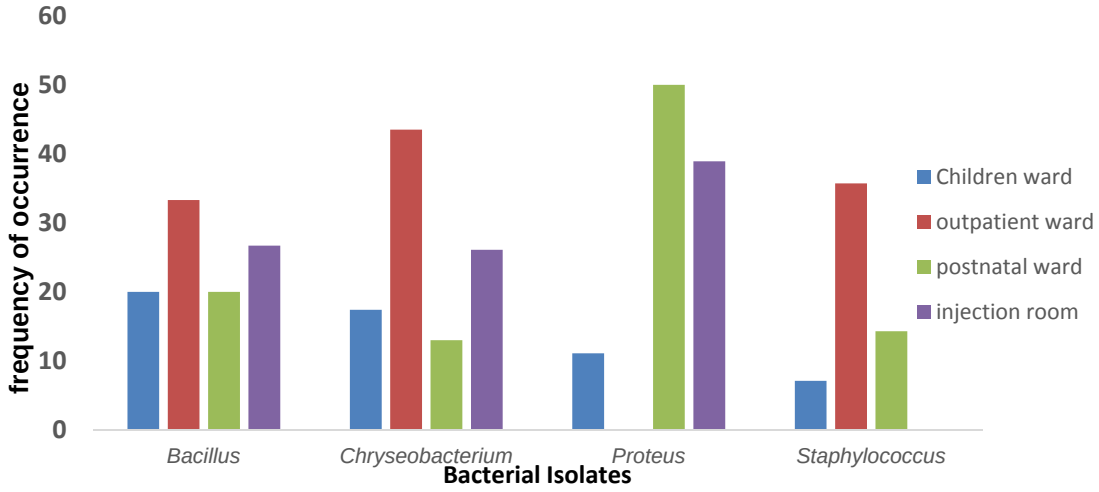

Fig 4: Frequency (%) of occurrence of bacteria in the four wards of Rumuigbo Primary Health Care.

With the exception of *Chryseobacterium* species in this study, *Bacillus, Proteus* and *Staphylococcus* species which occured in the indoor air of this current study have been identified in indoor air by previous studies to be associated with nosocomial infections [19, 20, 23, 27]. Wemedo and Robinson [3] have isolated *Bacillus* and *Staphylococcus* species from the indoor air of a government health centre which agreed with the current study. These microbes are mostly transmitted either through water, food or by person to person or through contact with noncritical surfaces. *Proteus mirabilis,* a gram-negative rod, is a normal inhabitant of the human gut and it is also found in water and soil. Its motile nature aids it in contaminating medical instruments and thereby enhances the chances of causing diseases. This pathogen accounts for 1-2% of urinary tract infections and 5% of hospital acquired urinary tract infections [28] *Proteus mirabilis* has been isolated as a contaminant of hospital equipment and was found to cause many ill health arising from gram negative bacilli [29]. Thus, there presence could be attributed to use of materials contaminated with the pathogen.

4. CONCLUSION

This study recorded high microbial loads which exceeds the suggested limits of 1000Cfu/m^3. The microorganisms identified in this study could be potential pathogens to the health of persons within these environments especially the patients and immune compromised persons. Contamination of hospital equipment is possible, thus proper sterilization of

equipment before use should be encouraged. Furthermore, proper ventilation should be a priority when setting up a health care institution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

ETHICAL APPROVAL

Ethical Approval was sought and obtained from the Ethical committee Rivers State Primary Health care board, Port Harcourt. The MOH granted the permission to conduct the research in the various wards.

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